28.7.2015 Interlab measurement

TUESDAY, 7/28

Markins in tubes: D1 L 28.7.2015 (J23101+I13504) and D3 L 28.7.2015 (J23117+I13504).

where the "L" means "Ligated"

Did the ligation for the restricted parts of J23101,I13504 and J23117 creating D1 (J23101+I13504) and D3 (J23117+I13504). Kept all enzymes and buffer.Made the following DNA-mixtures for ligation:

D1: AHD1+AHD4+AHD5

Table1

DNA sample	Volume of insertion (ul)
AHD1 R (J23117)	5,084477143
AHD4 R (I13504)	7,136864
AHD5 R (pSB1C3)	5,0

D3: AHD3+AHD4+AHD5:

Table2

DNA sample	Volume of insertion (ul)
AHD3 R (J23117)	5,084477143
AHD4 R (I13504)	7,136864
AHD5 R (pSB1C3)	5,0

Added the right amounts of DNA, 2ul of 10x T4 DNA ligase buffer and 0,2 ul T4 DNA ligase to each tube and balanced total volume to be 20 ul with nuclease-free water. After the addition of ligase, there was 20 min pause when the tubes were in ice before starting incubating 20 mins at 22C. The ligase was inactivated at 65C for 10 mins and put on ice.

Made the transformation into TOP10 for two plasmids: Ligated D1 and D3.
Added 50 ul of ice cold compenents cells into pre-chilled 2ml tube and 2 ul of resuspended DNA to the same tube, and mixed with pipette gently. Liquid was transparent and there was some moisture on the tube walls. The cells were incubated on ice for 30 mins. The tubes were put in a water bath (42C) for 60s. After the heat shock the cells were on ice incubation for 5 minutes. Incubated the cells at 37C for 1 hr with shaking (230 RPM). At this point there seemed to be growth in tubes, because the liquid turned to be bleary.

Two petri disher were labeled for each transformation with the transfer volumes of 50 µl and 100 µl, 4 of them with chloramphenicol (D1 and D3). The incubation of plates (37C, 230 RPM) started at 17:30 for overnnight cultivation.

29.7.2015 Interlab measurement

WEDNESDAY, 7/29

Plates for D1 and D3 were taken out from the 15h 30 min incubation. Couple colonies spotted from the yesterday's plates. However, during the overnight incubation the plates was set with the agar side directing at the bottom. The colonies were set under the UV light but no GFP-colonies were detected. The plates were put back to incubation at 10:00 and was taken out 16:00. Still, no colonies detected so the plates were discarded.

Also the newest restrictions of AHD1 R, AHD3 R, AHD4 R and AHD5 R (from 22.7.2015) was checked with the gel electrophoresis: Did a 1,2 % agarose gel with ETBR. Ran 2 ul samples with 0,4 ul LD of the restrictions. Used 2 ul Gene O'Ruler 1 kb ladder. Ran the gel for 25 minutes, 120 V. The results can be seen in Fig 1 where the samples 6-9 represents restrictions AHD1 R, AHD3 R, AHD4 R and AHD5 R. Its seems that AHD4 and AHD5 were succesfully restricted as the samples contained the right pieces. AHD1 and AHD3 are missing the 35 bp pieces or it can't be seen in the gel extraction. Nevertheless, AHD1 and AHD3 may need an another restriction. Furthermore, the amounts of AHD4 R and AHD5 R may be too low for further ligations so greater restricted stocks need to be also made.

Geldoc_2015-07-29_12hr_01min_cen_cex_agd1_ahd3_ahd4_ahd5.jpg

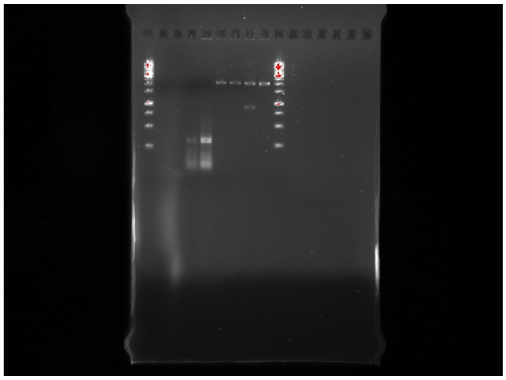

Fig 1. Gel electrophoresis results for AHD1, AHD3, AHD4 and AHD5.

30.7 Interlab

THURSDAY, 7/30

Markins in tubes: AHD1 R, AHD3 R, AHD4 R and AHD5 R with a current date.
Kept all enzymes and buffers on ice if the protocol didn't expect anything else. Added 5,0 ul 10x NEB CutSmart Buffer into the tube (material, dimensions, manufacturer. In order to get 250ng DNA for mixtures, the following volumes of purified plasmids were added to tube shown in Table 1 and the total liquid volume of the tube was balanced to be 50 ul with sterile water, after the enzymes were pipetted. Added 1,0ul of each restriction enzyme following Table 1. Mixed by pipetting and spinned the samples to get reagents in the bottom of tubes. Wasn't sure about the XbaI amount when the pippetting was proceeded so AHD4 will be checked later with the gel electrophoresis

Incubated samples at 37C for 30 min and inactivated the restriction enzymes at 80C for 20 mins. Stored the samples into -20C.

Table1

Sample	DNA (ng/µl)	DNA volume added (ul)	Ion-free water added (ul)	Restriction enzyme used
AHD1 (J23101)	93,5	5,34	37,66	EcoRI & SpeI
AHD3 (J23117)	48,8	10,24	32,76	EcoRI & SpeI
AHD4 (I13504)	124,6	4,02	38,98	XbaI & PstI
AHD5 (pSB1C3)	25,0	20,0	23,0	EcoRI & PstI